Coordination among Rate-limiting Steps of Glycolysis and Respiration in Intact Ascites Tumor Cells*

(Received for publication, July 11, 1966)

IN-YOUNG LEE,† ROBERT C. STRUNK, AND ELMON L. COE
From the Biochemistry Department, Northwestern University Medical School, Chicago, Illinois 60611

SUMMARY

The accumulation of lactate and phosphorylated glycolytic intermediates, as well as the variation in adenosine triphosphate, diphosphate, and 5'-monophosphate, were measured over the first 4 min after addition of 0.77 mm glucose to Ehrlich ascites carcinoma cells in 54 mm phosphate buffer (pH 7.4) at 37° and 30°. The rates of glucose flux through the phosphofructokinase step (V\text{PFK}) and the lactate dehydrogenase step (V\text{LDH}) were calculated and compared with each other and the rate of oxygen consumption (V\text{R}), measured by means of a bare, vibrating reed platinum electrode.

During the period from 10 to 90 sec or more after glucose addition, both V\text{R} and V\text{PFK} are linearly related to V\text{LDH}; at 37°, V\text{PFK} = 2.5 (V\text{LDH} - 1.1) and V\text{R} = 0.38 (V\text{LDH}), where velocities are measured in micromoles per ml of cells per min; at 30°, V\text{PFK} = 4.1 (V\text{LDH} - 1.3) and V\text{R} = 0.32 (V\text{LDH}). These results suggest a common controlling mechanism for all three rates.

The velocities are also approximately linear functions of esterified phosphate concentration. Since the increase in esters such as fructose diphosphate probably occurs at the expense of intracellular inorganic phosphate, this observation could be consistent with control of the rates by inorganic phosphate. The kinetic data do not identify either ADP or inorganic phosphate as the major controlling agent, but they do indicate a close coordination among the initial segment of glycolysis, the terminal segment of glycolysis, and respiration which is not fully explainable in terms of variations in either the ADP or phosphate levels in the whole cell.

The two major sites apparently controlling the sequence of glycolytic enzymes in intact Ehrlich ascites carcinoma cells are the combined glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase system (1, 2) and the phosphofructokinase (3, 4). Rate control of both has been attributed to either inorganic phosphate (1, 3) or adenosine diphosphate (2, 4) although the glyceraldehyde 3-phosphate dehydrogenase rate may be influenced also by the coupled lactate dehydrogenase reaction rate (4). The respiratory rate, initially accelerated along with glycolysis by addition of high concentrations of glucose, may depend on the availability of ADP (5). Hence, the interactions of the initial and final glycolysis segments with each other and with respiration probably depend on a coordinating mechanism involving both ADP and inorganic phosphate.

Inorganic phosphate has been shown to be a major factor in rate coordination within glycolysis by Uyeda and Racker (6, 7) although ADP involvement was not precluded. The ADP level has been definitely correlated with rates of phosphofructokinase, respiration, and lactate dehydrogenase in intact cells during the activated glycolysis and respiration period (4). Detailed studies of the relationships between respiratory rates and the initial and final glycolysis segments are undertaken herein. These rates are found to correlate linearly with each other during the activated stage of respiration and glycolysis immediately after glucose addition.

METHODS

Tumor Preparation—A hypotetraploid strain of Ehrlich ascites carcinoma cells was grown for 7 to 11 days in Swiss white mice and was prepared and incubated in a 54 mm phosphate buffer solution (phosphate-Locke, pH 7.3 to 7.4) as described previously (8, 9).

Experimental Procedures—One milliliter of aerated tumor suspension (15 to 25% of cells, by volume) was added to 5 ml of buffer, and after 60 sec, 0.5 ml of 10 mm glucose in buffer was also added. At accurately timed intervals after glucose addition, 3 ml of ice-cold 14% perchloric acid was rapidly blown into the suspensions. The perchloric acid extract was neutralized with 20% KOH and left at 5° overnight; after removal of the potassium perchlorate, these extracts were used directly for analyses involving enzymes. Additional filtrates were prepared from these with Ba(OH)\text{2} and ZnSO\text{4} for use in the lactate determination (4).

The Gilson Medical Electronics Oxygraph, model K, a polarographic device containing a bare, vibrating reed platinum cathode, was used for respiration studies as described previously (10, 11).
One milliliter of buffer was added to the chamber followed by 0.20 ml of the same tumor suspension used in the glycolysis experiment; after 60 sec, 0.10 ml of glucose solution was added and the recording was continued for 5 or 6 min.

Estimation of Metabolites—Glucose was estimated by means of a commercially prepared glucose oxidase-peroxidase-chromogen combination (Glucostat) (4). Glucose 6-phosphate, fructose 1,6-diphosphate, dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, α-glycerophosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, pyruvate, ADP, and ATP were determined by oxidation or reduction of pyridine nucleotides in the presence of specific enzymes, also described previously (4). Fructose 6-phosphate was estimated by adding phosphohexose isomerase after glucose 6-phosphate dehydrogenase to the system used for glucose 6-phosphate determination. AMP was estimated by adding myokinase and ATP to the system used for ADP determination. Lactate was determined by the method of Barker and Summerson (12) on barium-zinc filtrates. An enzymatic method (13), used in a few experiments, gave results close to those obtained with the colorimetric method.

Oxidized pyridine nucleotide was estimated by the fluorometric method of Jacobson and Astrachan (14).

Materials—All the nucleotides and most other biochemicals were obtained from Sigma in the purest available form. A highly purified barium salt of 2,3-diphosphoglyceric acid was obtained from Schwarz BioResearch; barium-silver phosphoenolpyruvate was obtained from Calbiochem. Water was distilled and deionized.

All the enzymes, except hexokinase, were secured from Sigma in crystalline form. Myokinase, α-glycerophosphate dehydrogenase, and phosphoglyceric acid mutase from Boehringer Mannheim Corporation, New York, were also used in some experiments. Hexokinase was Sigma type III.

RESULTS

Typical Metabolic Patterns—Table I summarizes the conditions used in the experiments described below. Most experiments were conducted at 37°, although a few at lower temperatures are also included for comparison. Since the patterns of glycolysis and respiration described previously (4, 15) were obtained at 30°, glycolytic patterns from a representative 37° experiment at a glucose concentration of 0.77 mm are given in Fig. 1. Variations in adenine mononucleotide levels in three representative experiments are summarized in Fig. 2. Data presented in Fig. 1 represent changes from the initial endogenous levels, whereas those in Fig. 2 are the actual concentrations.

As shown in Fig. 1, glucose-6-P increases rapidly for about 5 sec but then continues to rise gradually to a maximum at 45 to 60 sec. Glucose utilization slows as glucose-6-P approaches a maximum, indicating the possible involvement of a product inhibition of hexokinase (4, 6). There is a rapid drop in the ATP level to a minimum concomitant with the sharp increase in the ADP level to a maximum during the period of rapid glucose-6-P accumulation (Fig. 2). All three nucleotide levels then change gradually toward the endogenous level and reach a steady state around 60 sec. Fructose 1,6-di-P reaches a maximum at 45 to 60 sec and decreases slowly thereafter, and
lactate accumulation continues at a reduced rate after this period. The sequence of changes in the ADP level is reproducible, but the AMP curve exhibits considerable variation, following ADP in Experiment A and changing in the opposite direction in Experiment B (Fig. 2). In all cases the level of AMP is lower than either ADP or ATP, however.

The levels of fructose-6-P, glyceraldehyde-3-P, monophosphoglycerate, and phosphoenolpyruvate are low (0 to 0.2 μmole per ml of cells) and variations are not pronounced.

The correspondence between the fructose-1,6-di-P maximum and the re-establishment of steady state levels of ADP and ATP, and the correlation between the rapid period of fructose-1,6-di-P accumulation and the increase in ADP are observations consistent with an ATP inhibition of phosphofructokinase released by increasing ADP level [4].

Relationship between Velocities of Lactate Dehydrogenase and Phosphofructokinase Reactions—For ease of comparison, both velocities are calculated in terms of glucose equivalents, as described previously [4]. The velocity of lactate dehydrogenase (VLDH) is taken as half the rate of lactate accumulation, and the phosphofructokinase velocity is calculated from the summed rates of accumulation of products subsequent to the phosphofructokinase step

\[ V_{PFK} = \frac{d}{dt} (\text{fructose-1,6-di-P}) + \frac{1}{2} \frac{d}{dt} (\text{dihydroxyacetone-P + glyceraldehyde-3-P + P-glycerate + P-enolpyruvate + pyruvate + lactate}) \]

In practice, the rate of accumulation of each intermediate is estimated separately and the rates are then summed. Usually fructose-1,6-di-P, dihydroxyacetone phosphate, and lactate account for over 90% of accumulated products. This approach for calculating velocities from measured accumulation in intact cells assumes that the exogenous glucose added to the cells goes directly to lactate via glycolysis without major contributions from endogenous reserves. In most cases, this assumption seems warranted since the sum of the measured products comes close to the glucose consumed.

In a given experiment, the levels of glycolytic intermediates were plotted with time, as shown in Fig. 1, and point slopes of the smoothed curves were estimated at a series of times after glucose addition. Slopes were usually taken at 10, 20, 30, 45, 60, 120, 180, and 240 sec. The variation in VPFK with VLDH in Experiments A, B, and C is illustrated in Fig. 3. The highest points for all three experiments correspond to values obtained 10 sec after glucose addition. Line S represents the relationships between VPFK and VLDH under conditions of steady state glycolysis (VPFK = VLDH), whereas Line C corresponds to the condition where glycolytic ATP synthesis will support glycolytic ATP utilization (VPFK = 2VLDH). The observed line falls above Line C at values of VLDH greater than 5.6, indicating that some non-glycolytic source of ATP is required above this point. The observed line does not pass through the origin but crosses the abscissa at VLDH = 1.1.

Due to the accumulations of fructose-1,6-di-P and dihydroxyacetone phosphate, VPFK and VLDH are not directly proportional to each other and VPFK becomes less than VLDH when the fructose-1,6-di-P accumulation declines and the value of the term, d/dt (fructose-1,6-di-P), becomes negative (see equation for VPFK, above). Values obtained at high glucose levels but lower temperatures, (not shown in the figure) extrapolate to a VLDH of 1.3 and show a distinctly greater slope than the 37° line in Fig. 3, resulting in an intersection with Line C at a lower VLDH (2.6).

Relationship between Velocities of Lactate Dehydrogenase and Respiration—Rates of respiration were estimated directly from the slopes of Oxygraph tracings, as described previously (4, 10, 15). The relationships between Vr, the rate of respiration in micromoles of O2 consumed per min per ml of cells, and VLDH at 37° and room temperature are given in Figs. 4 and 5, respectively. Line R gives the relationship expected if the rate of ATP synthesis remained constant and glycolysis were operating under steady state conditions; as glycolysis increases, respiration would be depressed by a proportional amount. In actual fact, both VR and VLDH increase in a proportional manner during the early periods after glucose addition. As in Fig. 3, the highest points represent the earliest times. Respiration is stimulated when VLDH is greater than 5.0, corresponding to the point at which VPFK exceeds Line C in Fig. 3, an observation consistent with presumed ADP respiratory control. At values of VLDH where a glycolytic steady state is being established (about 1.8; see Line S, Fig. 3) most values of VR fall below Line R in Fig. 4, indicating a decrease in the net rate of ATP synthesis.

The relationship between VR and VLDH at lower temperatures (Fig. 5) indicates a progressive decrease in slope and in the VLDH at which respiratory stimulation begins. The point at which respiration exceeds the endogenous rate at 30° (VLDH = 2.7) corresponds to the point at which VPFK exceeds Line C in these experiments. Contrary to the data at 37° (Fig. 4) glycolysis approaches a steady state at a value of VLDH above Line R at 30 and 25°. The observed lines in Figs. 4 and 5 approach the Line R at VLDH values of 2.7 and 1.3 and at VR values of 1.0 and 0.43 at 37° and 30°, respectively, indicating...
that the rates of both respiratory and glycolytic ATP synthesis at a steady state are approximately doubled as the temperature increases from 30 to 37°. It is of interest that at all temperatures $V_{\text{PFK}}$ approaches $V_{\text{LDH}}$ at 1.7 to 1.8 whereas $V_R$ reaches Line R at different $V_{\text{LDH}}$ values as the temperature changes. The fact that glycolysis has internally established a steady state ($V_{\text{PFK}} - V_{\text{LDH}}$) before the $V_R - V_{\text{LDH}}$ line approaches Line R at 30° may account for the fact that the $V_R - V_{\text{LDH}}$ relationship stabilizes nicely at Line R at this lower temperature.

**Effect of Glucose Concentration on Intermediate Accumulation**—A series of experiments at glucose concentrations of 0.154 and 0.077 mM, not described in detail, were also conducted at 37°. Fig. 6 shows that lactate, fructose-1,6-di-P, and ADP accumulated 5 and 10 sec after glucose addition increase with glucose concentration but that glucose-6-P remains constant. The constant glucose-6-P level at different glucose concentrations indicates that the effect of increasing glucose concentration is more complex than a simple mass action effect on the glycolytic intermediates. The result is in accord with the report of Wu (3) that glucose utilization rate depends on glucose concentration below 1.2 mM, but in conflict with our previous conclusion (16) that lactate accumulation rate is independent of glucose concentration. This discrepancy is considered in "Discussion."

**Relationships between Enzymatic Rates and ADP Level**—Fig. 7 presents $V_{\text{LDH}}$ and $V_R$ as functions of the ADP level in Experiments A, B, and C (see Table I). In the case of $V_R$ (Fig. 7, lower) the initial, endogenous rates are included for comparison. The five open symbols for Experiments A and C and all points for Experiment B represent values at 10, 20, 30, 45, and 60 sec after glucose addition. The broken arrows to the solid symbols for Experiments A and C represent the shift observed after 60 sec; individual points after 60 sec fall along the arrow and approach the solid point between 120 and 180 sec. This shift is caused by a continuing decline in $V_R$ and $V_{\text{LDH}}$ during the secondary slight rise in ADP (Fig. 2).

During the 1st min, both $V_R$ and $V_{\text{LDH}}$ are linear functions of the ADP level, but they approach proportionality only in Experiment A. The extrapolation of the lines for Experiments B and C to the abscissa indicates that if ADP is the controlling factor, not all is available to the enzymes concerned. It is of interest that the initial (prior to glucose addition) $V_R$ points for Experiments B and C fall closer to the proportional line for Experiment A than to the lines through the other B and C points.

The observed linearity (Fig. 8) of $V_{\text{PFK}}$ with ADP is implied by the relationship of $V_{\text{PFK}}$ and $V_{\text{LDH}}$. The approximate linearity between $V_{\text{LDH}}$ and the ADP:ATP ratio and between $V_{\text{PFK}}$ and the square of the ADP:ATP ratio described earlier for Experiment E (4) also hold for Experiments A, B, and C as shown in Fig. 9. This points up the fact that the experimental results are not sufficiently precise to define the exact mathematical relationship between the velocities and nucleotide levels. The functions of ADP and ATP used previously (4) were chosen to approximate proportionality as closely as possible; the more varied results obtained in the later experiments presented here illustrate that no one function of ADP and ATP will consistently give a proportional relationship, although several different functions will approximate a linear relationship. For these reasons most considerations in the present paper are
restricted to the simplest of the possible functions, i.e. the ADP concentration.

Relationships between Enzymatic Rates and Esterified Phosphate—Inorganic phosphate has also been implicated in the control of glycolysis (1, 3), at least at low intracellular phosphate concentrations (9). In a high phosphate buffer, changes in intracellular phosphate are technically difficult to measure. However, penetration of inorganic phosphate into the cells under the conditions used appears to be relatively slow so that the change in intracellular inorganic phosphate over short time intervals may be approximated by the phosphate esterified, that is, \( P_i = C - P_{\text{est}}, \) where \( C \) is the concentration of \( P_i \) initially available inside the cell and \( P_{\text{est}} \) is the esterified phosphate. In Fig. 10 \( V_{\text{LDH}} \) and \( V_R \) are given as functions of the phosphate esterified in the forms of glucose-6-P, fructose-1,6-di-P, dihydroxyacetone-P, glyceraldehyde-3-P, P-glycerate, P-enolpyruvate, and ATP.

Fig. 7. Velocities of lactate dehydrogenase and oxygen consumption as a function of ADP level at 37°C. Data taken from Experiments A to C (Table I). Upper, glucose flux through lactate dehydrogenase (\( V_{\text{LDH}} \)); open symbols represent the first 60 sec; at later times the points fall along the arrows and approach the solid symbols. Lower, rate of oxygen consumption (\( V_R \)); circled symbols represent the initial rate, prior to glucose addition; other symbols as in upper. ADP is given in micromoles per ml of cells.

Fig. 8. Velocity of phosphofructokinase as a function of ADP level at 37°C. Data from Experiments A to C (Table I). Symbols used as described in Fig. 7.

Fig. 9. Velocities of lactate dehydrogenase as a function of the ADP:ATP ratio \( (ADP/ATP) \) and phosphofructokinase as a function of the square of the ADP:ATP ratio \( ((ADP/ATP)^2) \). Data are from Experiments A to C (Table I).

Fig. 10. Velocities of lactate dehydrogenase and oxygen consumption as a function of esterified phosphate at 37°C. Data taken from Experiments A to C (Table I). Ester phosphate represents the phosphate bound in the forms of fructose diphosphate, triose phosphate, and other glycolytic phosphates as well as ATP, given in micromoles per ml of cells. Symbols used as described in Fig. 7.

The same symbols as in Fig. 7 are used, and Experiments A, B, and C are represented. During the 1st min a linear relationship between \( V_{\text{LDH}} \) or \( V_R \) and ester phosphate occurs, followed by a shift, as with ADP. Were phosphate the controlling agent, the direction of the shift precludes penetration of extracellular inorganic phosphate as a cause. The extrapolated values between 10 and 14 \( \mu \) moles per ml of cells is within the 20 to 30 \( \mu \) moles measured internally. The initial endogenous values for \( V_R \) all fall below the lines (Fig. 10, lower) indicating that if
inorganic phosphate does control respiration, its availability must be increased on entry of glucose.

**DISCUSSION**

**Possible Errors Introduced by Glycolytic Intermediates from Endogenous Sources**—The various rate calculations presented above are based on the innocent belief that glucose passes simply through glycolysis and accumulates finally as lactate without drains or contributions by alternate pathways. Generally, this belief is defensible because the sum of the glycolytic products usually comes within 5% of the glucose consumed (compare Reference 4) but there are occasional exceptions. In Experiment A, for example, as much as 3 pmol of glucose per ml of cells in glucose equivalents may have come from endogenous sources. Some of this deviation may result from experimental error, but other evidence also indicates the existence of endogenous sources. A parallel experiment was run with Experiment A in which identical conditions were used in the presence of 50 μM oxamate. Despite the fact that this level of oxamate should be sufficient to block lactate dehydrogenase (17), a transitory accumulation of lactate occurred and was nearly equivalent to the excess glycolytic product accumulation in the unpoisoned cells. Moreover, the discrepancy between glucose utilization and product accumulation was about the same in the oxamate-poisoned system. How such endogenous contributions might affect the estimates of \( V_{LDH} \) and \( V_{PFK} \) depends entirely on how and where they feed into glycolysis; entry from glycogen, for example, would have no effect on the rate estimations after hexokinase.

In this regard, it is of interest that Kharchenko and Seitz (18) have found not only the enzymes for synthesizing glycogen in Ehrlich ascites cells but also reserves of glycogen equivalent to about 1 μmole of glucose per ml of cells. Whatever the source of the excess products in Experiment A, it appears to have little influence on the relationship between \( V_{PFK} \) and \( V_{LDH} \) calculated from the product accumulation (cf. Fig. 3).

**Interpretations of Linear Relationships**—Glycolysis can be divided into two major segments with respect to the rates observed during the "activated" period 10 to 90 sec after glucose addition. The "head" segment includes hexokinase, phosphofructokinase; the "tail" segment includes glyceraldehyde-3-P dehydrogenase, lactate dehydrogenase, and all the enzymes in between. Within either segment, all enzymes operate at nearly the same rate, although the two segments need not be equal in rate. Aldolase and triose-P isomerase represent an intermediate segment which lags behind the head but exceeds the tail segment (4). The tail segment appears to be internally regulated by a tight coupling between the two dehydrogenases through pyridine nucleotide such that a factor affecting the rate of any enzyme within the segment will influence the rate of the entire segment. Because of these characteristics a single enzymatic velocity may be taken as representing the velocity of the entire segment; hence \( V_{LDH} \) is an index of the velocity of the tail segment, just as \( V_{PFK} \) indicates the velocity of the whole head segment, and correlations of metabolite levels with either velocity do not necessarily imply a specific effect on the lactate dehydrogenase or phosphofructokinase.

From the linear relationships in Figs. 3 to 5, 7, 8, and 10 and 11 a variety of slopes and intercepts can be obtained; some of these are summarized in Table II, with the use of the general formula \( y = ax + b \). The close coordination among \( V_{PFK} \), \( V_{LDH} \), and \( V_R \) implicates a concerted mechanism acting on several control points which may parallel the situation in muscle described by Cori (19), although the activation in the tumor cell depends on a sudden introduction of free glucose rather than on an excitatory phosphorolysis of endogenous glycogen. Prior to glucose addition in washed ascites cells the levels of all phosphorylated glycolytic intermediates are low (<0.1 μmole per ml of cells); compare zero time points in Reference 4) and no glycolytic activity can be detected from accumulation of lactate or the intermediates.

The constancy of ATP synthesis in the Ehrlich ascites cells demonstrated by Quastel and Bickis (20, 21) implied that the "phosphate cycle" concept of Lynen (22) and the theory of control by ATP hydrolysis products of Johnson (23) were applicable. Both ADP (2, 4, 5, 8) and inorganic orthophosphate (1, 3) have variously been advocated as the agents controlling glycolysis and respiration either as rate-limiting substrates or, more recently, as allosteric activators of phosphofructokinase (3, 4). From the relationships between \( V_{PFK} \), \( V_{LDH} \), and \( V_R \) and both ADP and esterified phosphate evident in Figs. 7, 8, and 10, it is apparent that either ADP or Pi control of the head and tail segments of glycolysis and respiration could be consistent with the data in the present work. As indicated above, another potential controlling factor, AMP, did not exhibit a consistent pattern and plots of the velocities against AMP level gave no consistent trend toward an increase or decrease in velocity with AMP. If either ADP or Pi is the major controlling agent, some compartmentalization must be postulated to explain the breaks in the curves and the existence of an ADP intercept. It is striking that both \( V_{LDH} \) and \( V_R \) as functions of ADP show

<table>
<thead>
<tr>
<th>Table II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear relationships among velocities, ADP, and ester phosphate</td>
</tr>
</tbody>
</table>

Experiments are given in Table I. The constants, \( a \) and \( b \), give the relationship between \( x \) and \( y \) in the general equation \( y = ax + b \), as estimated from the line drawn in the cited figure.

<table>
<thead>
<tr>
<th>( y )</th>
<th>( x )</th>
<th>Experiments</th>
<th>Temperature</th>
<th>( a )</th>
<th>( b )</th>
<th>Figures</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{PFK} )</td>
<td>( V_{LDH} )</td>
<td>A-C</td>
<td>37</td>
<td>5.0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>37</td>
<td>11.4</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>37</td>
<td>7.7</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>( V_R )</td>
<td>( V_{LDH} )</td>
<td>A-C</td>
<td>37</td>
<td>2.10</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-E</td>
<td>37</td>
<td>3.30</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F-H</td>
<td>37</td>
<td>3.25</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>( V_{PFK} )</td>
<td>( V_{LDH} )</td>
<td>A</td>
<td>37</td>
<td>10.6</td>
<td>0.14</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>37</td>
<td>28.0</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>37</td>
<td>21.0</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>( V_{LDH} )</td>
<td>( P_{est} )</td>
<td>A</td>
<td>37</td>
<td>-0.67</td>
<td>13.7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>37</td>
<td>1.16</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>37</td>
<td>-0.79</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>( V_R )</td>
<td>( P_{est} )</td>
<td>A</td>
<td>37</td>
<td>-0.283</td>
<td>13.7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>37</td>
<td>-0.315</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>37</td>
<td>-0.323</td>
<td>10.4</td>
<td></td>
</tr>
</tbody>
</table>

\( P_{est} \) represents esterified phosphate.
the same ADP intercept (Table II) and that both shift in the
same direction after 60 sec. If ADP is controlling, the same
pool is available to both respiration and glycolysis, and the
change in compartmentalization involves a simultaneous decrease
in the availability of ADP to both respiration and glycolysis.
A similar argument may be applied to phosphate control because
of the comparable 60-sec shifts in Fig. 10; these could not be
attributable to an influx of extracellular phosphate (see above).
Such a change in distribution of ADP or phosphate is quite
distinct from the movement out of a glycolytic compartment or vice versa, implied by some of the earlier hypoth-
theses.

The foregoing consideration of the possible roles of both ADP
and inorganic phosphate in the control of glycolysis is complic-
ed by the remarkably consistent relationship between \( V_{PFK} \)
and \( V_{LDH} \) shown in Fig. 3, despite the variety of slopes and in-
tercepts obtained when either velocity is plotted as a function
of ADP (Figs. 7 and 8) or esterified phosphate (Fig. 10). Not
only does the relationship between \( V_{PFK} \) and \( V_{LDH} \) appear
nearly identical for Experiments A, B, and C, but the relation-
ship continues to hold true for well over 60 sec, after the original
linearity of velocity with either ADP or esterified phosphate
begins to break down. It should be emphasized that the inter-
pretations made in terms of ADP or phosphate control are ex-
pressed in the following form: "if ADP (or phosphate) is the
controlling agent, then one must conclude that . . . ." The fact
that the correlations among the rates themselves are more con-
sistent than the correlations between the rates and any of the
supposed controlling agents demands that we re-examine some
of the basic premises of the current hypotheses about metabolic
control mechanisms. It is conceivable that ADP and phosphate
are only indirectly related to the actual control mechanism and
may not be tightly coupled with glucose phosphorylation at low concentrations.

Factors Controlling Earliest Periods of Glycolysis—The parallel
increase in lactate, fructose-1,6-di-P, and ADP accumulation with increasing glucose concentration during the first 5 to 10
sec after glucose addition (Fig. 6) raises several interesting ques-
tions. For one, we were previously (16) unable to find a clear
trend toward increasing initial rates of lactate accumulation as
glucose concentration increases, whereas in Fig. 6 such a trend
is evident. This may be attributable in part to an improvement
in the precision of the lactate determinations and in part to a
change in the strain of tumor used from hyperdiploid (16) to
hypotetraploid. The range of lactate accumulation rates illus-
trated in Fig. 6 (4.2 to 10.2 pmoles per ml of cells per min) is
essentially the same as that given previously, however (3.6 to
10.2), and is less than the glucose concentration range.

The observed parallelism between lactate and fructose-1,6-
di-P along with the constancy in glucose-6-P clearly implies
that glucose concentration influences the rates of both the glyco-
lytic segment from glucose to fructose-1,6-di-P and the segment
from triose-P to lactate, but that the influence operates in such
a fashion that glucose-6-P accumulation is restricted to 0.2
\( \mu \) mole. Glucose concentration is near the \( K_m \) for hexokinase
(0.01 to 0.1 mM, compare Reference 25); hence, substrate con-
centration could be limiting. Although increasing glucose
concentration could accelerate hexokinase, it must also acel-
erate phosphofructokinase by an equivalent amount, or a greater
accumulation of glucose-6-P would also be observed. A more
exact determination of rates of accumulation at less than 5 sec
after glucose addition might help to explain this phenomenon.

Reliability of Correlations—In conclusion, it should be empha-
sized that the correlations described are approximate and may
be subject to certain systematic errors. It is possible, for example,
that diffusion of oxygen into the solution in the electrode cham-
ber might tend to lower the apparent \( V_R \) at later times, although
trials with a chemical model system (alkaline pyrogallol) in-
dicate that such diffusion is negligible compared to \( V_R \) under
the usual conditions of the experiment. Even assuming accuracy
in the data, the scatter is sufficient to make exact relationships
uncertain. In Table II, the slope and intercept of the \( V_R \)
- \( V_{LDH} \) line in Fig. 4 (one of the worst examples) are given as 0.38
and 0 \( \mu \) mole per ml of cells per min, respectively, although it
might be possible to construct lines with slopes ranging from 0.32
to 0.44 and intercepts up to 1.5 or so and still remain in the
general region of the early points. The much less scattered points from comparable data at lower temperatures (Fig. 5)
suggest that the line should pass through the origin, and the
mean line, including points from all three experiments up to 60
sec, would come close to the origin, but the possible errors must
be borne in mind.

Acknowledgments—The authors gratefully acknowledge the
skillful technical assistance of Helen Burson, the critical appraisal
of the manuscript by Mary H. Coe, and the interesting discus-
sions of the data with Jagneswar Saha, Sharon Cleland, and
Dr. Ernst Helmreich.

REFERENCES
2. Hess, B., in B. Wright (Editor), Control mechanisms in
respiration and fermentation, The Ronald Press Company,
8. Ibsen, K. H., Coe, E. L., and McKee, R. W., Biochim. Bio-
20, 1599 (1960).
334 (1941).
14. Jacobson, K. B., and Asterlachan, L., Arch. Biochim. Bio-
17. Noyoa, W. B., Winne, A. D., Glaud, A. J., and Schilling,


Coordination among Rate-limiting Steps of Glycolysis and Respiration in Intact Ascites Tumor Cells
In-Young Lee, Robert C. Strunk and Elmon L. Coe


Access the most updated version of this article at http://www.jbc.org/content/242/9/2021

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/242/9/2021.full.html#ref-list-1